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Pollinator adaptation and the evolution of floral nectar sugar composition

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Key words: asterids, fructose, glucose, pollination syndrome, phylogenetic conservatism, phylogenetic constraint, sucrose

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ABSTRACT

A longstanding debate concerns whether nectar sugar composition evolves as an adaptation to pollinator dietary requirements or whether it is 'phylogenetically constrained'. Here we use a modeling approach to evaluate the hypothesis that nectar sucrose proportion (NSP) is an adaptation to pollinators. We analyze ~2,100 species of asterids, spanning several plant families and pollinator groups (PGs), and show that the hypothesis of adaptation cannot be rejected: NSP evolves toward two optimal values, high NSP for specialist-pollinated and low NSP for generalist-pollinated plants. However, the inferred adaptive process is weak, suggesting that adaptation to PG only provides a partial explanation for how nectar evolves. Additional factors are therefore needed to fully explain nectar evolution and we suggest that future studies might incorporate floral shape and size and the abiotic environment into the analytical framework. Further, we show that NSP and PG evolution are correlated – in a manner dictated by pollinator behavior. This contrasts with the view that a plant necessarily has to adapt its nectar composition to ensure pollination but rather suggests that pollinators adapt their foraging behavior or dietary requirements to the nectar sugar composition presented by the plants. Finally, we document unexpectedly sucrose-poor nectar in some specialized nectarivorous bird-pollinated plants from the Old World, which might represent an overlooked form of pollinator deception. Thus, our broad study provides several new insights into how nectar evolves and we conclude by discussing why maintaining the conceptual dichotomy between adaptation and constraint might be unhelpful for advancing this field.

Introduction

Understanding the evolution of floral rewards is central to understanding the evolution of plant-pollinator interactions. Nectar is the main floral reward provided by the vast majority of modern angiosperms (70-80%, extracted from Heywood *et al.*, 2007) but despite coadaptation between plants and their animal pollinators being thought to be one of the key mechanisms responsible for angiosperm evolution and floral diversification (Stebbins, 1970; Harrison *et al.*, 1999), our understanding of the evolution of floral nectar remains poor. On the one hand, it has been suggested that the chemical composition of nectar, an aqueous solution comprising primarily the monosaccharides glucose and fructose and the disaccharide sucrose (Baker & Baker, 1982), is as an adaptation to the nutritional constraints or preferences of a plant's pollinator(s) as well as to flower morphology (Baker & Baker, 1975, 1982, 1983a). On the other hand, the sugar composition of floral nectar has been found to be relatively invariable within plant clades, with any interspecific

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differences being independent of the plants' main pollinators (e.g. van Wyk, 1993; Galetto *et al.*, 1998; Nicolson & van Wyk, 1998; Galetto & Bernadello, 2003; Rodríguez *et al.*, 2014). These seemingly contradictory perspectives call into question our understanding of what drives the evolution of floral rewards and, in particular, floral nectar.

Early studies documented convergence in the sugar composition of nectar among plants pollinated by the same pollinator group (PG; Baker & Baker, 1982, 1983a). Various groups of insects (bees, wasps, butterflies, moths and some groups of flies) and several groups of vertebrates (e.g. hummingbirds, sunbirds, honeyeaters, phyllostomid and pteropodid bats) are obligate nectar feeders and, by visiting flowers regularly, often also act as pollinators (referred to as specialists; Fleming & Muchhala, 2008). In addition, a surprising variety of unspecialized vertebrates and insects (songbirds, geckos, mice, kinkajous, short-tongued flies and some groups of beetles) are known to feed on nectar occasionally (referred to as generalists; e.g. van Tets & Nicolson, 2000; Nicolson, 2002; Hansen *et al.*, 2006; Johnson & Nicolson, 2008). These animals lack special morphological adaptations to feed on nectar but are still known to be effective pollinators. However, their nutritional requirements differ from those of nectar-feeding specialists with respect to sugar concentration (in solution) or composition (relative contribution of sucrose and the two hexoses, fructose and glucose, to total sugar content). While most specialist PGs, such as moths, bees or hummingbirds, prefer nectar with a high sucrose proportion (Nicolson *et al.*, 2007; Johnson & Nicolson, 2008), nectar-feeding bats prefer a low sucrose proportion (Baker *et al.*, 1998). Similarly, sucrose-rich nectar cannot be digested as efficiently by, or is even toxic to, some generalists (Martínez del Río, 1990; Martínez del Río *et al.*, 1992). Therefore, it has frequently been proposed that interspecific differences in nectar sugar composition and concentration, especially in nectar sucrose proportion (NSP), reflect adaptations to the dietary requirements of different PGs (e.g. Heynemann, 1983; Martínez del Río *et al.*, 1992; Baker *et al.*, 1998).

In addition, there is a long-recognized correlation between floral shape and NSP, such that deep or tubular flowers tend to have high NSP and shallow flowers tend to have low NSP (high hexose proportion; Percival, 1961). This correlation has been attributed to the environment, because open flowers run greater risk of nectar evaporation, rendering them useless to pollinators (Baker & Baker, 1983a). Hexose solutions have higher osmolarity, and therefore lower evaporation rates, than sucrose solutions and this is thought to explain the correlation between shallow flowers and nectars with a high proportion of hexose (Nicolson *et al.*, 2007). However, high-hexose nectars also tend to require more water. In Mediterranean regions there is a predominance of tubular flowers with high-sucrose nectars, suggested to be the result of selection against high-hexose nectars in a warm, dry climate (Nicolson *et al.*, 2007; Petanidou, 2005). This correlation reflects the physical constraints of
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sugar solutions themselves but it is also thought to constitute evidence for adaptation to pollinators because a plant will only be regularly visited by efficient pollinators, whose requirements of sufficiently dilute, easily accessible nectar are fulfilled (Nicolson *et al.*, 2007).

Given the obvious adaptive advantage of a good fit between floral traits and animal pollinators, it is surprising that more recent studies of plant species pollinated by different PGs have failed to find NSP values characteristic of the individual PGs (e.g. van Wyk, 1993; Galetto *et al.*, 1998; Nicolson & van Wyk, 1998; Galetto & Bernadello, 2003). Instead, similar NSP has been recorded for closely related species in the same plant family (e.g. in Gesneriaceae, Proteaceae and Scrophulariaceae), and in aloes and relatives NSP has been found to be conserved within but not among genera, irrespective of PG (e.g. van Wyk *et al.*, 1993; Nicolson & Thornburg, 2007; Rodríguez-Riaño *et al.*, 2014). These findings have led to the suggestion that there is a “phylogenetic constraint” (*sic*) on the adaptation of NSP to pollinators (Galetto & Bernadello, 2003; Thornburg, 2007; Rodríguez-Riaño *et al.*, 2014). For example, it has been suggested that the differences in NSP between plants pollinated by hummingbirds and passerine birds might be because they belong to different plant clades rather than due to any innate differences in the requirements of the pollinators themselves (Nicolson & Thornburg, 2007).

Considerable attention has been paid to the question of what causes interspecific differences in the relative proportions of fructose, glucose and sucrose in nectar. To date, the debate has generally been centered on a perceived dichotomy between adaptation to pollinators and phylogenetic constraints (e.g. Schmidt-Lebuhn *et al.*, 2006; Nicolson *et al.*, 2007). We are not aware of any explicit, biological mechanism generating the constraint being proposed and believe that focus on the perceived dichotomy between adaptation and conservatism may have hampered progress into understanding of how floral nectar evolves. In general, phylogenetic conservatism or constraint¹, is often invoked to explain the lack of variation among close relatives or the tendency of closely related species to retain their ancestral state over time (e.g. Wiens & Graham, 2005; Cooper *et al.*,

¹ Phylogenetic constraint, phylogenetic conservatism and phylogenetic inertia are here used interchangeably to describe a pattern of evolutionary stasis, lack of variation among close relatives, and over time, or retention of ancestral traits. We are aware that use varies among authors and may even refer to the process of failing to change, adapt to some factor or occupy some habitat or region (Wiens & Graham, 2005; Ackerly, 2009; Cooper *et al.*, 2010; Wiens *et al.*, 2010; Losos, 2011; Cook & Crisp, 2012).

2010; Wiens *et al.*, 2010; Crisp & Cook, 2012). However, this conveys only that there is limited trait variation among close relatives, not what the causal explanation for the observed pattern might be (Westoby *et al.*, 1995; Blomberg & Garland, 2002; Losos, 2011). In the case of nectar, documentation of phylogenetic conservatism in sugar composition suggests limited variation among closely related plant species, irrespective of their pollinators, but provides no insight into how floral nectar evolves, nor does it constitute evidence for or against adaptation (Leroi *et al.*, 1994; Blomberg & Garland, 2002; Ackerly, 2003, 2004; Crisp & Cook, 2012). Both stasis and change can result from both adaptive and non-adaptive processes; e.g., stabilizing selection provides an adaptive explanation for the pattern of stasis and retention of the ancestral state does not mean that the trait in question is not an adaptation to something (Westoby *et al.*, 1995; Ackerly, 2004; Losos, 2011; Hansen, 2014). For instance, if a correlation between NSP and PG were rejected, the hypothesis that NSP evolves as an adaptation to PG might be rejected, but that would not preclude that nectar sugar composition was an adaptation to something else, say, corolla shape and size (Nicolson, 2002; Witt *et al.*, 2013). Alternatively, a non-adaptive hypothesis might be favored if change in NSP were found to be stochastic with respect to *any* environmental variable to which it were hypothesized to be an adaptation; e.g., if it were found to be drifting according to the allometric constraints of floral shape and size or the physical constraints of the environment.

Thus, mechanistic explanations do not require inference of conservatism and can be distinguished using comparative methods, by setting up testable hypotheses to be evaluated in a model-comparison framework (Hansen, 1997; Butler & King, 2004). Here, we use this approach to evaluate the hypothesis that nectar sugar composition, in particular NSP, is an adaptation to pollinator preferences. We compile a dataset that is unprecedented in scope for this purpose, with nectar sugar composition, pollinator and phylogenetic data broadly sampled for the asterids, a major angiosperm clade of about 80,000 species (Bremer, 2009), including the carrots, daisies, heathers and mints. We define the sugar composition of nectar as the trait and PG as the environment. We then test for a correlation between the trait and the environment, such that there is convergence of the trait in relation to the environment (i.e., convergence of nectar sugar composition for plants pollinated by the same PG; Leroi *et al.*, 1994; Ackerly, 2004) and such that an adaptive shift in the trait is associated with a shift in function (i.e., a new pollination syndrome; Hansen, 1997; Butler & King, 2004). We also explore the nature of the hypothesized adaptation by asking whether the trait or the environment changes first (Pagel, 1994; Ackerly, 2004)?

Pollinators are thought mainly to be sensitive to the proportion of total sugar constituted by sucrose (NSP), while the proportion fructose (NFP) and glucose (NGP) are thought not to provide reliable evidence of pollination syndromes (Baker *et al.* 1998). Based on this, we analyzed the

proportional content of each sugar in turn to evaluate support for the following hypotheses. **H1:** Nectar sucrose is an adaptation to pollinators. This would be supported if a correlation between NSP and PG cannot be rejected, if there is evidence that adaptation to the environment is rapid and accurate and if change between NSP and PG is correlated, such that change in PG (the environment) precedes or accompanies change in NSP (the trait). **H2:** Nectar fructose and glucose are not adaptations to pollinators. This would be supported if a correlation between the trait (NFP or NGP) and the environment (PG) is rejected or if there is evidence that shifts in the trait in relation to the environment are slow and achieved by stochastic, rather than adaptive, change.

Material and Methods

Nectar data

We compiled nectar sugar composition data for asterids using published records and, focusing on previously neglected taxa (e.g. from the Old World tropics), collected and analyzed new nectar samples for this study. We focused on the proportional content of the three main nectar sugars (fructose, glucose and sucrose) and chose the asterids because of their diversity in floral structure, geographical range and pollinators. Furthermore, a wealth of published data on pollination ecology is available for this clade, accessed by searching for publications in ISI Web of Knowledge and Google Scholar using the terms “nectar sugar composition” and “nectar sugar content”. In contrast to nectar volume and total sugar concentration, which are heavily influenced by water availability and microclimate, nectar sugar composition is relatively constant within plant species (Baker & Baker, 1983a; Schwerdtfeger, 1996; Torres & Galetto, 1998; Nicolson & Thornburg, 2007). The relatively low level of intraspecific variation that has been documented is mainly due to differences among individuals and only to a small degree due to differences between populations in the wild or between plants growing in the wild or in botanical gardens (e.g. Freeman & Wilken, 1987; Vickery & Sutherland, 1994; Janza *et al.*, 1995; Gijbels *et al.*, 2014). We further reduced the potential for variation in nectar sugar composition by collecting nectar only from young flowers to minimize the impact of bacteria and yeasts, which are able to transfer sucrose to hexoses (Nicolson & Thornburg, 2007). We collected nectar between September 2007 and August 2011 in the field in Europe, Southeast Asia and South America and in botanical gardens. We used glass capillaries to collect nectar from at least three young flowers per plant species. The minimum volume of nectar collected per species was 1 µl (Morrant *et al.*, 2009). We placed the nectar on filter paper, air dried it and stored it in silica gel for up to a few months to prevent microbial decomposition of the sugars. Overall sugar concentration (%) and composition (relative proportions of fructose, glucose and

sucrose) were determined using standard protocols (SI text). The proportional content of each sugar was normalized by logit transformation prior to analysis.

Definition of pollinator groups

Due to the large variety of concepts for defining PGs, these were considered carefully. Because plants that are mainly pollinated by unspecialized nectar-feeding birds (generalists) contrast in their morphology and physiology with plants that are mainly pollinated by obligate nectar-feeding birds (specialists; Johnson & Nicolson, 2008), we treated specialized and unspecialized bird species as different PGs. Generalist passerine and non-passerine birds that feed on nectar, as well as on larger amounts of fruits, seeds or insects, are referred to as unspecialized (e.g. orioles [Oriolidae], bulbuls [Pycnonotidae], white-eyes [Zosteropidae], Hawaiian honeycreepers [Drepaniidae] and flowerpeckers [Dicaeidae]; Amadon, 1950; Stiles, 1981; Johnson & Nicolson, 2008; del Hoyo *et al.*, 2008). Some of these birds (thrushes, starlings, and mockingbirds; families in the Muscicapidae) lack the ability to digest sucrose (Martínez del Río, 1990), while others (waxwings; Bombycillidae) are able to digest sucrose but not as efficiently as they are able to digest hexoses (Martínez del Río *et al.*, 1992). In contrast, the bird species we refer to as specialized, such as New World hummingbirds (Trochilidae) and Old World sunbirds (Nectariniidae), sugarbirds (Promeropidae), and some small-bodied genera of honeyeaters (Meliphagidae) and lorikeets (Psittacidae; Hopper & Burbridge, 1979; Pyke, 1980; Stiles, 1981; Tjørve *et al.*, 2005; Johnson & Nicolson, 2008), take most of their energy from nectar and are able to digest sucrose efficiently. Behavioral differences between specialized nectar-feeding birds in the Old and New Worlds are associated with different characteristics of their food plants. Therefore, we treated specialized, nectar-feeding birds from the Old and New Worlds as separate PGs.

Several concepts for insect pollination systems exist. The distinction between plants pollinated by short-tongued bees and butterflies short-tongued bees or long-tongued bees (e.g. Baker & Baker, 1975, 1982, 1983a, 1983b) can be difficult to detect in nature (Schwerdtfeger, 1996). Also, the details of the pollinators of these plants are largely unknown. Instead, we adopted the two categories developed by Schwerdtfeger (1996): 1) typical bee-pollinated, often zygomorphic flowers are referred to as bee-pollinated and 2) relatively small, open flowers, providing access to the nectar for a wide range of insects (e.g. small bees and butterflies, wasps, flies and beetles) are referred to as generalist insect-pollinated. Similarly, we recognized a butterfly-pollinated and a moth-pollinated group, mainly distinguishing the species pollinated during the day and night, respectively (Schwerdtfeger, 1996). This contrasts with the various groups of plants pollinated by different groups

of butterflies recognized by some authors (Baker & Baker, 1975, 1982, 1983a, 1983b). In the end, we defined nine pollinator groups: generalist insects, bees and wasps, specialized flies, butterflies, moths, New World bats, unspecialized birds, specialized Old World birds and hummingbirds.

We obtained information on each asterid species' affiliation to one pollinator group from the same publications used to extract the published data on nectar sugar composition. For the newly generated data (~30% of species) we used pollinator observations from the literature (e.g. Jäger & Rothmaler, 2011) or our own field observations. For species where the pollinator is unknown we used categories based on floral morphology, coloration and scent (Faegri & van der Pijl, 1978) because this is known to be a reliable method for identifying the most effective, main PGs of a plant (Fenster *et al.*, 2004; Rosas-Guerrero *et al.*, 2014). For example, brightly colored, often red, scentless flowers with long, relatively wide corolla tubes, pending stigmas and stamens without a landing platform were scored as pollinated by specialized Old World birds or, in the Americas, hummingbirds. Flowers with a landing platform, long, narrow corolla tubes and bright colors were scored as butterfly-pollinated.

Phylogenetic information

Published, DNA-based phylogenies for most species in the nectar dataset are available but the DNA markers used differ among studies, rendering data coverage for individual genes highly incomplete. Therefore, we used phylogenetic information from published studies for all sampled asterid species to generate a summary phylogeny. At the higher taxonomic level we referred to Smith *et al.* (2011) and the phylomatic webpage (Webb *et al.*, 2009). For resolution among and within genera we used published phylogenies based on multiple genes (supplementary data, S1; Abrahamczyk *et al.*, 2016) to manually place species using Mesquite 2.74 (Maddison & Maddison, 2009). Polytomies were randomly resolved 100 times to generate a set of 100 trees that reflect some, shallower phylogenetic uncertainty (e.g. within genera). The final set of trees comprised 2063 species, for which both nectar and PG data were available.

Many trait evolution analyses rely on phylogenetic distances (e.g. all models founded in Brownian motion; e.g. Pagel, 1999; Thomas & Freckleton, 2012) and since the assembled tree lacked branch-length information three approaches for providing branch lengths were explored (figure S1). The most realistic distribution of branch lengths, representing the results of numerous independent studies (table S1), was obtained by setting all branch lengths to 1.0 and then scaling the tree height to absolute time using 110 published age constraints (table S1) in pathd8 (Britton *et al.*, 2007).

We devised a series of models (table 1) that allow change in nectar sugar to depart in various ways from the null expectation of constant change that is random in direction (Brownian motion, BM; Schluter *et al.*, 1997). The first departure allowed the rate of change in nectar sugar to vary over time and among clades ('differential rates'), independently of PG. The number of rate shifts was increased incrementally until no better models were found (i.e., all shifts supported by $\Delta AICc \geq 6$ were identified; Thomas & Freckleton, 2012). For practicality and to avoid inferring spurious shifts the minimum clade size for detecting shifts was set to 100.

Next a series of Ornstein-Uhlenbeck (OU) models (Hansen, 1997; Butler & King, 2004) was fitted. These allow change in nectar sugar to be directional, toward one or more optimal values, at a rate dictated both by the strength of the pull (rate of adaptation) and the rate of (stochastic) change toward the optimum. The first model allowed directional change toward a single optimum value (OU-1), the second allowed the mean optimum to differ among species in different clades (OU-Clades; with clades defined as having uniform rates change in the differential rates analysis; 16 putative optima; see Results) and the third allowed the mean optimum to differ among species pollinated by different PGs (OU-PG; 9 putative optima). More complex models in which the adaptive pull or stochastic rate may vary among optima (Beaulieu *et al.*, 2012) were explored, but with 9 or 16 putative optima such models soon become highly parameter rich. Although the current dataset is large, not all putative optima are represented by many data points (table S8), and preliminary findings indicated that one or more parameters could not be reliably estimated. This resulted in a suboptimal model overall. Results for these more complex models are therefore considered unreliable and are not reported. Instead, to further test our hypotheses, three simpler models based on the results of the OU-PG model (see Results) were devised for NSP only (table 1): one in which the mean optimum was allowed to differ between specialist and generalist PGs (OU-2), one in which rates of stochastic change toward those optima may also differ (OU-2V) and one in which the strength of the pull toward those optima may also differ (OU-2A).

All models were fitted using maximum likelihood (ML) in R (R Development Core Team 2014) and model fit was compared using sample-size corrected AIC values (AICc; Burnham & Anderson, 2002). The BM and 'differential rates' models were fitted using 'transformPhylo.ML' in motmot (Thomas & Freckleton, 2012) and the OU models were fitted using OUwie (Beaulieu & O'Meara, 2015). Ancestral states, which determine the phylogenetic distribution of each putative selective regime, were determined using the equal-rates model in the 'ace' function of ape (Paradis *et al.*, 2004) for PG and manually for plant clade.

Model fitting in a continuous-time Markov framework: correlation analyses

The results of the above analyses suggested that NSP (but not NFP or NGP) is an adaptation to PG (see Results). To further explore this we tested for correlated change between the NSP and PG. We treated each variable as binary, with PG coded as 'specialist' (bats, specialized flies, bees and wasps, specialized birds, butterflies, moths and hummingbirds) or 'generalist' (generalist insects and unspecialized birds) and NSP as 'high' (>0.45) or 'low' (≤ 0.45 , the 84th percentile [mean + 1 standard deviation]) for all generalist-pollinated plants; table S4). Next we used two continuous-time Markov models for discrete traits to test whether change in one trait depends on the state of the second trait (Pagel, 1994; Pagel & Meade, 2006). The first model states that rates of change in one trait are independent of the other trait (i.e., 0 \rightarrow 1 and 1 \rightarrow 0 transitions occur at the same rate irrespective of whether the second trait is in state 0 or 1). The second model states that rates of change in one trait are dependent on the other trait (i.e., 0 \rightarrow 1 and 1 \rightarrow 0 transitions in one trait may differ depending on whether the second trait is in state 0 or 1). There are eight possible transitions in the dependent model and four in the independent model (table 2). Models were fitted with 10 ML iterations for each of the 100 trees using the Discrete functions in BayesTraits V2.0 (Quad Precision version for large trees; available from www.evolution.rdg.ac.uk/BayesTraits.html) and fit was compared using a likelihood ratio (LR) test with 4 degrees of freedom (d.f.) on each tree.

Robustness of results to phylogenetic uncertainty and scale and compared to simulated data

Three sets of analyses were performed to test the effect of 1) phylogenetic uncertainty (by comparing the phylogenetic signal of each nectar sugar and pollinator data across the set of 100 trees), 2) phylogeny alone (by comparing results of the differential rates analysis to those for simulated data) and 3) phylogenetic scale (by comparing best-fitting models across the asterids as a whole to those for a set of less inclusive clades). Details of these analyses are provided in the SI text.

Results

Variation in nectar sugar composition in relation to pollinator group

The nectar sugar composition dataset comprised 2,116 species and subspecies of asterids, representing 660 genera, 55 families, and 13 of the 16 orders. Roughly two thirds of these data (1480 species) were taken from the literature (see supplementary data, S2; Abrahamczyk *et al.*, 2016). Data for the remaining 636 species were generated for this study (577 species sampled from Botanical

Gardens, 59 from the wild, with samples from the wild spanning a range of families and pollinator groups [supplementary data, S2; Abrahamczyk *et al.*, 2016]). A ternary plot shows separation of nectar sugar composition along the sucrose axis, with plants pollinated by generalists being found at lower NSP than plants pollinated by specialists (figure 1A, figure S2). Only hummingbird-pollinated plants show a skew with respect to the two hexoses, being shifted toward fructose. There is a high degree of variation in the NSP of species pollinated by most PGs, especially by specialized flies, Old World birds and butterflies, and much overlap among them (figure 1B). In particular, we documented unexpectedly sucrose-poor nectar for some species pollinated by Old World specialized birds that have nectar with a low overall sugar concentration (figure S3).

Best-fitting evolutionary models for NSP, NFP and NGP

The best-fitting model for NSP was OU-PG and for NFP and NGP differential rates (table 1). Two independent optima were inferred for NSP: low NSP for plants pollinated by generalist birds and unspecialized insects and high NSP for plants pollinated by all other PGs studied here (figure 2). This model is a much better fit to the data ($\Delta\text{AICc} \geq 783$) than any of the other BM-based models. However, the parameter estimates from this model suggest that the adaptive process is weak (table 3). The stationary variance, a measure of the rate of drift relative to the strength of selection, is very high ($\sigma^2/2\alpha = 2.5 \times 10^5$) and the phylogenetic half-life, defined as the time needed to evolve half the distance from the ancestral state to the trait optimum, is extremely long ($\ln[2]/\alpha = 4.0 \times 10^5$ Ma). The simpler models (OU-2, OU-2V and OU-2A), although a worse fit to the data (third best overall; table 1), confirm that generalist-pollinated plants are evolving toward a lower NSP optimum than specialist-pollinated plants and yield phylogenetic half-life estimates of ~ 10 Ma, i.e., suggesting an adaptive process in which species are closer to their optimum and that is achievable within about 10% of the age of the asterids (table S2).

The second best model for NSP was the differential rates model, in which 15 rate shifts were identified (figure 3, table S3). Seven shifts were slowdowns and eight speedups; several shifts were nested and most shifts were found in Ericales, where rates generally increased in shallower clades, or Gentianales + Lamiales, where rates generally decreased in shallower clades. Most clades identified correspond to, or almost to, major named clades. This could be an artifact of the relatively sparse sample analyzed here or, alternatively, suggests correspondence between evolutionary processes and named clades that we are only beginning to be able to detect (Smith *et al.*, 2011; Humphreys & Barraclough, 2014). Fifteen rate shifts were also found under the overall best model for NFP ($\Delta\text{AICc} \geq 354$; tables 1, S3). Half of these occur at exactly the same node as for NSP and the other half occur

only one or a few nodes away. Under the overall best model for NGP ($\Delta AICc \geq 122$), there was support for 11 rate shifts. Again, shifts tended to occur at the same nodes as for NSP, NFP or both. The pattern of slowdowns and speedups was the same for all three sugars: decreases tended to occur in Gentianales and Lamiales and speedups in Ericales. The second best model for both NFP and NGP was OU-Clades.

Correlation analyses: nectar sucrose proportion and pollinator group

When NSP and PG are coded as binary variables, there is significant dependency in the data ($P < 0.0001$, Fisher's Exact test, two-tailed; table S4). Specialist PGs are more likely to be associated with high than low NSP and very few generalist PGs are associated with high NSP (specialists are three times more likely to pollinate high-NSP flowers and generalists six times more likely to pollinate low-NSP flowers). This association does not hold the other way round: both high-NSP and low-NSP flowers are more likely to be pollinated by a specialist than a generalist pollinator (68 and 4 times more likely, respectively).

More formally, the model of dependent evolution between NSP and PG could not be rejected for any of the 100 trees ($P < 0.0001$, LR tests with 4 d.f.). A comparison of rate parameter estimates reveals the nature of this dependency: a high-NSP, specialist-pollinated plant is more likely to change into a low-NSP, specialist-pollinated plant than into a high-NSP generalist-pollinated plant ($q_{13} > q_{12}$; 98% of trees; table 2). This suggests that nectar shifts first from the ancestral state. In addition, shifts from a specialist to generalist PG are more likely in a low sugar background ($q_{34} > q_{12}$; 94% of trees) and shifts from high to low NSP are more likely in a generalist PG background ($q_{24} > q_{13}$; 96% of trees). Rate estimates for the reverse transitions were indistinguishable.

Robustness to phylogenetic uncertainty and scale and compared to simulated data

Estimates of phylogenetic signal were constant across the set of 100 trees for all three sugars and PG (SI text, table S5). Thus, our findings are robust to some phylogenetic uncertainty. The results of the differential rates analysis differed for empirical and simulated data (figure S4). Thus, overall, the pattern of rate increases and decreases is not an artifact of the phylogeny and NSP is evolving differently to expectations for a neutral trait. However, some shift positions were recovered for both empirical and simulated data (SI text; table S3) and these should be interpreted with caution because they can apparently be recovered with any trait. Finally, analysis of less inclusive clades revealed a strong effect of phylogenetic scale. For NSP, the findings for the asterids overall were confirmed but,

in addition, differences among specialist PGs as well as among clades were found (table 3, S6, figure S5). Evidence for adaptation, e.g. as indicated by short phylogenetic half-lives relative to overall tree height, was much stronger for clades analyzed separately than for asterids overall. These findings were largely mirrored for NFP and NGP, thus contradicting results across the asterids as a whole for these two sugars (SI text, tables S6, S7, figure S5).

Discussion

Unexpected variation in nectar sugar composition for many pollinator groups

Based on the results of Baker and Baker (1982), we expected plants pollinated by one PG to have converged on the optimum NSP for that PG. Instead, we found high variability in the NSP of plant species pollinated by most PGs, most notably by specialized flies, bees and wasps, butterflies and specialized Old World birds (figure 1). The variability for these insect-pollinated plants may be because of different preferences in males and females (Rusterholz & Erhardt, 1997, 2002), different requirements for different pollinator subgroups (Petanidou, 2005; Goldblatt & Manning, 1999) or different optimal NSP values in different plant clades (explored further below). The variability for plants pollinated by specialized Old World nectarivorous birds probably requires a different explanation. Nectarivorous birds are known to prefer sucrose-rich nectars with a high overall sugar concentration but experiments have shown that they prefer nectar composed of hexoses if the overall sugar concentration of the nectar is low (5%; Brown *et al.*, 2010). This is because the birds are sensitive to small changes in osmolarity of the nectar and hexose solutions have a higher osmolarity than sucrose solutions (Nicolson & Fleming, 2003). Our finding, that about a third of the species pollinated by Old World nectarivorous birds produce nectar with low overall sugar concentration (< 20%) that is extremely sucrose poor (NSP < 0.20; figure S3), corroborates the results of Brown *et al.* (2010) and suggests that this phenomenon may be more widespread in nature than previously thought. It is possible that this represents an alternative, energy-saving pollination strategy, which is deceptive to the pollinators because of the comparatively energy-poor nectar they are rewarded with. Similar deception strategies probably occur in many plant species belonging to several genera both within and outside the asterids (e.g. van Wyk *et al.*, 1993; Nicolson & van Wyk, 1998; this study). Further studies are needed to test this hypothesis.

We tested the hypothesis that NSP is an adaptation to PG (e.g. Heynemann, 1983; Martínez del Río *et al.*, 1992; Baker *et al.*, 1998). At first glance, our results suggest that this hypothesis cannot be rejected: we found strong statistical support for convergence on different NSP optima among different PGs and for correlated evolution between NSP and PG. But to what extent do the details of our findings constitute evidence for NSP being an adaptation to PG?

The best model for NSP evolution overall is where NSP is allowed to evolve toward different optima for each of the nine PGs studied here (table 1). Inspection of the inferred optima reveal that they in fact converge on only two independent optimal values, one for plants pollinated by generalists and one for plants pollinated by specialists (figure 2). Lack of differences among plants pollinated by most obligate nectar feeders implies that the NSP requirements of the individual specialist PGs, while being generally high, are indistinguishable. This could be one reason why previous studies (van Wyk, 1993; Nicolson & van Wyk, 1998; Galetto *et al.*, 1998; Galetto & Bernadello, 2003) have failed to detect significant differences in NSP among (specialist) PGs. Further inspection of the best-fitting model reveals a weak adaptive process (table 3). The phylogenetic half-life is unrealistically long, suggesting that species are far from their optimum and unlikely ever to reach it (Hansen, 1997; T.F. Hansen, pers. comm.). The stochastic variance is also very high, suggesting that any adaptive evolution toward the NSP optima is overwhelmed by stochastic movement and that a large amount of the residual variance of the model is not explained by the two optima. One interpretation of such a model is that it does not represent adaptation to peaks in the adaptive landscape in the strict sense but rather an adaptive trend of increasing divergence from the ancestral state (Hansen, 1997, 2012; T.F. Hansen, pers. comm.). Such an interpretation has been invoked for body size evolution in relation to dietary niches in cetaceans and habitat in monitor lizards (Slater *et al.*, 2010; Collar *et al.*, 2011). For nectar evolution this would suggest slow movement toward low NSP in generalist-pollinated plants and high NSP in specialist-pollinated plants. However, the optima inferred from these models tend to lie outside currently occupied ranges, perhaps themselves therefore representing unreachable adaptive peaks. The optima inferred for NSP approach the limits of the occupied range but are not unrealistic (table 3). Another possible interpretation, therefore, is that NSP is evolving as an adaptation to other, unmeasured variables (Labra *et al.*, 2009; Hansen, 2012), for example corolla shape and size (Nicolson, 2002; Witt *et al.*, 2013), perhaps as a function of the abiotic environment (Petanidou, 2005; Nicolson *et al.*, 2007). A third possible interpretation is that NSP is drifting, bounded by the allometric constraints of floral shape and size, which in turn could be correlated with PG. Such an explanation has been invoked for body shape evolution in three-spined stickleback (Voje *et al.*, 2013). Without formally incorporating

these variables into the hypothesis-testing framework, these alternatives cannot be distinguished but results from the simpler OU models (OU2, OU2A and OU2V; table S2) do offer some additional insight: these models suggest a much stronger adaptive process, at the coarse level of generalist PGs versus specialist PGs. This supports the interpretation of adaptation rather than that of drift. However, because the simpler models are a much worse fit to the data, they do not capture the entire story. The more complex model is a far superior fit to the data but one that nevertheless explains less of the residual variance. Together, these results suggest that PG is an important component of NSP evolution but it does not act directly as hypothesized here – other factors are needed to fully explain how NSP evolves.

Is nectar sucrose proportion the trait or the environment?

The dependency in the NSP and PG data coded as binary variables suggests that a flower is more likely to be pollinated by a specialist pollinator, irrespective of its NSP (table S4). In contrast, a specialized nectar feeder is more likely to pollinate a high-NSP flower and a generalist feeder is more likely to pollinate a low-NSP flower. This asymmetry is explained by the finding that NSP is the first to shift from the ancestral state of high NSP/specialist PG ($q_{13} > q_{12}$; table 2); i.e., changes in NSP may occur without a simultaneous or preceding change in PG. Once variation in NSP has been established, shifts from a specialist to generalist PG are more likely in lineages with low-NSP nectar and further shifts from high to low NSP are more likely in generalist-pollinated lineages. Thus, the evolution of NSP and PG is correlated in a way that means that certain shifts are more likely than others, but not in a way that requires simultaneous change, as would be expected under strict coevolution (Janzen 1980). The finding that NSP changes first suggests that the nature of the correlation is primarily dictated by pollinator behavior rather than vice versa. In other words, it suggests that pollinating animals “capitalize” on the NSP presented by the plant and that the plants do not necessarily adapt their NSP to the local pollinator guild (cf. “diffuse coevolution” of Janzen (1980)). This contrasts with the view that a plant must adapt its nectar composition to ensure regular visitation by efficient pollinators (Nicolson *et al.*, 2007). A conceptual consequence of this is that NSP should perhaps be treated as the environment and PG the trait – in order to be fully understood, the floral rewards-pollinator interaction might better be viewed from the opposite perspective to that often presented in the literature (and this study; Nicolson *et al.*, 2007 and references therein).

Different processes in different clades and for different sugars?

Analysis of less inclusive clades revealed several important insights. Evidence for adaptation was much stronger for individual clades than for asterids as a whole: an adaptive model could not be rejected for 12 out of 13 clades (table S6) and a much more rapid and precise adaptive process was inferred from these models than from the model for asterids overall (table 3). This supports the idea that, at the broadest scale, several factors are needed to explain nectar evolution, only one of which is adaptation to the broadly defined PGs analyzed here. Certain clades, however, revealed strong evidence for adaptation to PG in some clades and none at all in other clades. Multiple-optima models tended to corroborate the finding of independent optima for generalist and specialist PGs (figure S5) but also found differences among specialist PGs. One interesting example is the low-NSP optimum inferred for specialized nectarivorous birds, providing further support for the hypothesis of deception elaborated upon above. In contrast, single-optimum models revealed different optima in different clades, irrespective of the main PGs. A high optimum was inferred for the heather family (Ericaceae) and a low optimum for the daisy family (Asteraceae). Ericaceae are pollinated entirely by specialist PGs (bees and wasps, specialized OW birds and hummingbirds sampled here), while Asteraceae are pollinated by both specialist and generalist PGs (generalist insects, bees and wasps, butterflies and hummingbirds; table S8). Ericaceae tend to have tubular flowers and Asteraceae small, open flowers, suggesting that the different optima inferred for these two clades could be governed by floral shape and size, rather than the main PGs of each clade. These results further support both conclusions above that PG alone cannot explain how interspecific differences in NSP evolve and that the relationship between NSP and PG is perhaps not governed by adaptations of the plant but by pollinator behavior and dietary requirements.

Finally, these findings hold true not only for NSP but for NFP and NGP as well (tables S6, S7, figure S5). This contrasts with findings for asterids as a whole, where an adaptive model was rejected for these two sugars (table 1) and suggests either that pollinators *are* sensitive to the proportion of the two hexoses in nectar (*contra* Baker *et al.*, 1998) or, because our analyses were based on proportions, that the signal in NSP cannot be independent of that in NFP and/or NGP, even if different processes govern the relative proportions of each sugar. Future (experimental) work may shed further light on these alternatives.

Much of the historical debate surrounding the evolution of nectar sugar composition has centered on the dichotomy between adaptation to pollinator dietary requirements and there being a “phylogenetic constraint”, limiting the amount of variation that can accumulate within and among clades (Baker & Baker, 1975; Nicolson *et al.*, 2007). Indeed, results such as those above, of different processes operating in different clades and of clade-specific NSP optima that are independent of pollinator diversity, are likely to underlie some earlier claims of phylogenetic conservatism (Galetto & Bernadello, 2003; Thornburg, 2007; Rodríguez-Riaño *et al.*, 2014). However, although we found that adaptation to pollinators is not a sufficient explanation on its own, *phylogeny* is merely a depiction of patterns and cannot in itself constrain or explain anything (e.g. Losos, 2011). Many empirical studies have invoked both adaptation and conservatism in cases such as ours, where there is some evidence for adaptation, but where the specific hypothesis being tested leaves some observed variance unexplained (Ackerly, 2004; Cattin *et al.*, 2004; Escuerdo *et al.*, 2012; Hansen, 2014). However, in these studies, phylogenetic conservatism is not invoked as an alternative to adaptation but to describe a strong historical signal not directly related to the hypothesis being tested. In other words, conservatism is invoked in lieu of the full mechanistic explanation, just as appears to be the case in the nectar literature.

Given the longstanding clarity, far beyond the nectar literature, of the inadequacy of the adaptation/conservatism dichotomy (e.g. Leroi *et al.*, 1994; Blomberg & Garland, 2002; Ackerly, 2004; Losos, 2011), why does it persist? We suggest that there are several reasons.

i) As a result of (naïve) interpretation of phylogenetic patterns of trait similarity and divergence, often measured as phylogenetic signal (Losos, 2008; Cooper *et al.*, 2010; Crisp & Cook, 2012), as process. However, while it has long been clear that patterns cannot simply be read from phylogenies and interpreted as processes (e.g. Blomberg & Garland, 2002), the extent to which phylogenetic signal is disconnected from any underlying process has only become clear relatively recently (e.g. Revell *et al.*, 2008; Boucher *et al.*, 2014; Münkemüller *et al.*, 2015). Therefore the practice of inferring process from phylogenetic patterns remains.

ii) Because of the cladistic tradition of recognizing only autapomorphies as adaptations (Hansen, 2014). This means that a retained ancestral state (plesiomorphy) cannot be interpreted as an adaptation. The counter argument is that retained plesiomorphies must be adaptations for something or they would have succumbed to selection pressures to change (Losos, 2011; Hansen, 2014). Thus, retention of the ancestral state is a pattern that says nothing of its generating or maintaining evolutionary process.

iii) Because of the terminology used in the OU framework of adaptive models (“adaptation-inertia models”; e.g. Pienaar *et al.*, 2013). This use may certainly be perceived as conceptually confusing but inference of “inertia” over “adaptation” does not necessarily mean that the trait in question is not an adaptation at all, only that the specific hypothesis under study is rejected (Hansen, 2014). The trait may still be an adaptation to an environment that itself is evolving in a drift-like manner or to another, unmeasured variable that shows strong similarity among closely related species (Labra *et al.*, 2009; Hansen, 2012). Thus, although model inferences may be described as either “adaptation” or “inertia”, interpretation is not necessarily dichotomous.

iv) Due to the development of the conceptual frameworks for studying adaptation and conservatism as largely different fields. This is most likely historical: as one field was realizing the challenges involved in inferring adaptation in a comparative framework (Baum & Larsson 1991; Leroi *et al.*, 1994; Hansen, 1997), another, dedicated to detecting (niche) conservatism (Harvey & Pagel, 1991; Wiens & Graham, 2005; Crisp & Cook, 2012), was born. Increasingly, these fields make use of the same models (*cf.* e.g. Kozak & Wiens, 2010; Münkemüller *et al.*, 2015) but because they are developing in parallel (one is concerned with adaptation [Hansen, 1997; Butler & King, 2004; Beaulieu *et al.*, 2012], the other with a “failure to adapt” [Wiens *et al.*, 2010; Kozak & Wiens, 2010]), reconciliation of how adaptation and conservatism can be interpreted together has received much less attention than each phenomenon has separately (but see Ackerly 2003, 2004; Labra, *et al.*, 2009).

Conclusion

We present evidence that NSP is an adaptation to PG in asterids but, importantly, that this is not the whole story. Future studies may increase mechanistic understanding of how plant-pollinator interactions via floral rewards evolve by focusing on dense sampling of narrower clades, finer divisions of PGs and incorporating additional factors into the hypothesis-testing framework, along with consideration of how the floral nectar-pollinator interaction arises, without the need to invoke phylogenetic constraints or conservatism.

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Table 1. Models founded in Brownian motion compared for nectar sucrose proportion (NSP), nectar fructose proportion (NFP) and nectar glucose proportion (NGP).

Model	Hypothesis	Free parameters	AICc (NSP)	AICc (NFP)	AICc (NGP)
BM	Nectar sugar evolves independently of PG and is random in direction, with a mean change of zero and a rate of change that is constant over time and among lineages	$k = 2$ (root state and rate of change)	10539.4	8820.2	9901.5
Differential rates	As BM but the rate of change may vary over time and among lineages	$k = 2 + 2n$ (root state, n number of rate shifts and $n + 1$ number of rates)	9931.7	8004.3	9220.2
OU-1	Nectar sugar evolves independently of PG but is directional, toward a global optimum state; the strength of the pull toward and rate of (stochastic) change toward the optimum are constant over time and among lineages	$k = 3$ (rate of change, strength of pull [=adaptive change] and 1 trait optimum)	10160.5	8417.5	9398.0
OU-Clades	As OU-1 but directional change is toward several optima, which may differ among clades (defined as having their own rate of change in the differential rates analysis; Table S3)	$k = 18$ (as OU-1 and 16 putative optima)	10134.5	8357.8	9342.8
OU-PG	As OU-1 but directional change is toward several optima, which may differ among PGs; thus nectar sugar evolution is dependent on PG	$k = 11$ (as OU-1 and 9 putative optima)	9149.1	8395.8	9344.6
OU-2	As OU-PG but directional change is toward two optima, which may differ between generalist and specialist PGs	$k = 4$ (as OU-1 and 2 putative optima)	10096.3	—	—

OU-2V	As OU-2 but the rate of change may differ between optima	$k = 5$ (as OU-2 and 2 putative rates of stochastic change)	10098.2	–	–
OU-2A	As OU-2 but the strength of pull may differ between optima	$k = 5$ (as OU-2 and 2 putative rates of adaptive change [=pull])	10096.9	–	–

AICc values in bold denote the best-fitting model.

BM = Brownian motion; OU = Ornstein Uhlenbeck

Table 2. Definition of rate parameters compared in the correlation analyses.

Parameter	Evolutionary transition ¹	Estimate ² (median [95% CI])
<i>Forward shifts (0 -> 1)</i>		
q_{12}	Shift from specialist to generalist in high sucrose background	0.00077 (0.00048–0.010)
q_{13}	Shift from high to low sucrose in specialist background	0.018 (0.017–0.019)
q_{24}	Shift from high to low sucrose in generalist background	0.45 (0.015–3.1)
q_{34}	Shift from specialist to generalist in low sucrose background	0.0044 (0.0033–0.0058)
<i>Reverse shifts (1 -> 0)</i>		

q_{21}	Shift from generalist to specialist in high sucrose background	0.00 (0.00–1.51)
q_{31}	Shift from low to high sucrose in a specialist background	0.054 (0.050–0.058)
q_{42}	Shift from low to high sucrose in a generalist background	0.045 (0.0083–0.48)
q_{43}	Shift from generalist to specialist in low sucrose background	0.030 (0.022–0.043)

¹Eight transitions are possible under the dependent model because transition rates in one trait may vary depending on state of the second trait. In the independent model, transition rates in one trait are the same irrespective of the state of the second trait. Thus $q_{12}=q_{34}$, $q_{21}=q_{43}$, $q_{13}=q_{24}$ and $q_{31}=q_{42}$, and the independent model has four rate parameters.

²Rate estimates shown are summaries across the 100 trees; comparisons reported in the text were performed across each tree individually and cannot be read directly from this table.

Table 3. Parameter¹ estimates for the best-fitting OU model for asterids overall and for each of the less inclusive clades analyzed separately, where such a model could not be rejected (see Table S6).

Clade	Model ²	σ^2	α	θ – generalist insects	θ – bats	θ – bees and wasps	θ – specialized flies	θ – specialized Old World birds	θ – unspecialized birds	θ – butterflies	θ – moths	θ – hummingbirds	$t_{1/2}$ (th)	ν_1
Asterids	OU-PG	0.87	<0.001	<0.001	45.3	78.1	99.9	53.3	<0.001	15.6	100	99.8	4.0×10^5 (114)	2.5×10^5
Fouquieriaceae + Polemoniaceae + Primulaceae	OU-1	0.19	0.087	–	–	53.7	–	–	–	53.7	–	53.7	7.98 (67)	1.11
Core Ericaceae	OU-1	5.65	0.11	–	–	85.7	–	85.7	–	–	–	85.7	6.37 (49)	25.9
Core Asteraceae	OU-1	1.12	0.062	23.6	–	23.6	–	–	–	23.6	–	23.6	11.1 (39)	8.98
Core Solanaceae	OU-1	3.38	0.15	–	34.7	34.7	34.7	–	–	34.7	34.7	34.7	4.53 (28)	11.0
Gelsemiaceae + Gentianaceae + Apocynaceae	OU-1	1.80	0.057	–	82.4	82.4	82.4	–	–	82.4	82.4	82.4	12.1 (62)	15.7
Rubiaceae	OU-1	1.37	0.12	–	–	78.2	78.2	–	–	78.2	78.2	78.2	5.78 (56)	5.72
Gesnerioideae	OU-PG	23.4	9.98	–	36.2	88.5	–	–	–	–	–	77.8	0.070 (31)	1.17
Core Plantaginaceae	OU-PG	0.69	0.068	<0.001	–	57.8	–	–	–	–	–	74.8	10.2 (46)	5.07
Core Acanthaceae	OU-1	0.67	0.053	–	–	60.5	–	–	–	60.5	–	60.5	13.0 (34)	6.26
Ajugoideae + Lamioideae	OU-PG	81.9	9.74	–	–	82.4	–	28.8	–	74.2	–	91.8	0.071 (34)	4.20

Nepetoideae	OU-PG	15.5	5.59	8.16	–	70.0	83.9	–	–	–	–	78.1	0.12 (38)	1.38
Campanulaceae	OU-PG	0.35	0.057	–	6.30	23.3	–	–	1.15	–	–	77.7	12.2 (56)	3.09
Boraginales	BM	0.39	NA	–	–	NA	–	–	–	–	–	–	NA (57)	NA

¹ σ^2 is the rate of stochastic change (e.g. under drift or in relation to an unmeasured factor), α is the strength of the “pull” toward the selective optima (rate of adaptation) in Ma^{-1} , θ is the mean trait optimum, $t_{1/2}$ is the phylogenetic half-life in Ma with total tree height (th) in Ma in brackets ($\ln(2)/\alpha$; the time needed to move from the ancestral state to the new optimum) and v_y is the stationary variance ($\sigma^2/2\alpha$; the balance between the action controlled by α and that controlled by σ). Optimum values are shown as back-transformed mean estimates (% NSP; see figure S5 for variation around the mean).

²OU-PG is a model in which the mean optimum may differ among pollinator groups (PGs)³. Not all PGs are present in all clades (see Table S8) so the number of optima differs among clades.

Figure 1. Composition of nectar sugars and variation among pollinator groups. *A*: Ternary plot of the relative contributions of fructose, glucose and sucrose to the nectar sugar utilized by each pollinator group (PG). All groups separate along the sucrose axis with generalist pollinator groups tending toward lower nectar sucrose proportion (NSP; main plot: all data, $n=2116$; inset: group means; see figure S2 for details). NW = New World; OW = Old World. See main text for definitions of PGs. *B*: Distribution of NSP among plants pollinated by different PGs. Horizontal line = median, boxes = upper and lower quartiles, circles = outliers (defined as lying beyond $1.5 \times$ the interquartile range [Q3-Q1]). Outliers represent several plant families and are based on data sampled from both wild and cultivated plants.

Figure 2. Selective optima inferred for nectar sucrose content (NSP) in relation to pollinator group (PG) in asterids. The main differences are found between generalist and specialist PGs, with plants pollinated by generalist insects and unspecialized birds tending toward lower NSP optima than species pollinated by other PGs. See table 3 for details. Colors and symbols as in figure 1.

Figure 3: Phylogenetic position of rate shifts inferred in the differential rates analysis for nectar sucrose proportion (NSP). Branch colors show rates as exceptionally high (red) to exceptionally low (blue), through intermediate rates (yellow then green), compared to background rates (black). Major sampled orders are named. *The line not named (next to Dipsacales + Aquifoliales) is Apiales + Escalloniales + Bruniales. Clades at which rate shifts occur are numbered; details are provided in table S3. Based on a support measure (shift support) calculated from the likelihood of finding a shift at each node, three shifts should be treated with caution: core Solanaceae (node 2389, SS = 0.53), core Plantaginaceae (node 2276, SS = 0.85) and Gesnerioideae (node 3438, SS = 0.92). Shift support (SS) is $1 - f_{\text{exp}}$, where f_{exp} is the expected frequency of shifts at that node. The differential rates analysis for nectar glucose (NGP) and nectar fructose proportion (NFP) revealed similar results as for NSP (table S3).



